Pyramiding Quantitative Trait Locus (QTL) Alleles Determining Resistance to Barley Stripe Rust: Effects on Resistance at the Seedling Stage

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ABSTRACT

Durable disease resistance may be achieved by pyramiding multiple qualitative resistance genes in single genotypes and by using quantitative resistance (QR) genes. Quantitative trait locus (QTL) analysis tools can be used to find determinants of QR. Resistance QTL pyramids may also lead to durable resistance, and they provide independent validation of QTL effects and QTL interactions. We used molecular markers to identify allelic architectures at three previously mapped QTL conferring resistance to barley stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *hordei*) in a set of barley (*Hordeum vulgare* L. subsp. *vulgare*) doubled haploid (DH) lines. The three QTL are located on three different chromosomes. One parent contributed the resistance alleles at two QTL and another parent contributed the resistance allele at the third QTL. In this report, we focus on resistance at the seedling stage; resistance at the adult plant stage will be addressed in a future report. The DH population was phenotyped for resistance by means of four pathogen isolates that show different patterns of virulence on a set of differentials. We used molecular markers to infer the resistance QTL allele architecture of each DH line. There was no significant QTL × race interaction, although some DH lines showed differential responses to isolates. The effects and locations of two QTL, each tracing to a different parent, were validated. The third QTL did not have a significant effect on disease symptom expression. To maximize the probability of recovering the resistant phenotype, resistance alleles are necessary at both QTL.

Host pathogen resistance to biotic stresses can be classified as qualitative or quantitative. The former refers to Mendelian genes of large effect that clearly interact on a gene-for-gene basis with the pathogen, whereas the latter describes resistance that shows continuous variation and is usually incomplete in expression. The race specificity of quantitative resistance is still an unresolved question. Qualitative resistance can be measured as the reaction of either seedling or adult plants to inoculation, and its use for the development of new cultivars can be straightforward. The main problem with qualitative resistance is the lack of durability (Parlevliet, 1977). Quantitative resistance (QR) is often determined in the field and it is more likely to be durable (Parlevliet, 1989). The principal constraints to breeding for QR are that it requires extensive field testing at multiple growth stages and that the level of QR conferred by a single locus may not be adequate.

Pyramiding qualitative resistance genes with different race specificities has been proposed as a way to increase the likelihood of achieving durable resistance with qualitative resistance genes (Schaffer and Roelfs, 1985; Mundt, 1990). Singh et al. (2001) pyramided three genes conferring resistance to bacterial blight (caused by *Xanthomonas oryzae* pv. *oryzae*) in rice (*Oryza sativa* L.), providing a wider spectrum of resistance. Tabien et al. (2000) reported main effects and nonlinear interactions between multiple genes conferring resistance to rice blast (caused by *Magnaporthe grisea*) in a rice mapping population.

Pyramiding multiple QR alleles in single genotypes may also be an approach to increasing the level of resistance relative to that conferred by a single QR locus and multigenic QR may also lead to greater durability. However, data to support this hypothesis are lacking. Quantitative trait locus (QTL) analysis procedures have facilitated the dissection of QR, revealing that in some cases a significant proportion of the total variance in the expression of the resistance can be attributable to one locus or a few loci (Chen et al., 1994; Michelmore, 1995; Hayes et al., 1996; Young, 1996). However, information on the mechanisms underlying quantitative resistance is still very limited.

The QTL concept represented an important step forward in understanding traits showing quantitative variation (Doerge, 2002). Some plant breeders have embraced QTL mapping tools to increase selection efficiency via marker-assisted selection (MAS). However, QTL analysis has been shown to be subject to serious limitations. These include bias in QTL estimation, lack of studies validating QTL alleles in different genetic backgrounds, and few examples of successful MAS (Dekkers and Hospital, 2002).

In this paper, we present data from the barley: barley stripe rust (BSR) pathosystem that lead us to be reasonably optimistic about the application of QTL analysis and MAS to the development of disease-resistant genotypes. Stripe rust is an important disease of barley worldwide. It has been reported in the Americas since 1975, and in the USA since 1991 (Dubin and Stubbs, 1985; Marshall and Sutton, 1995). The population of BSR in the Americas was first described as race 24 and its variants (Dubin and Stubbs, 1985), but considerable variation has since been reported in pathogen isolates collected in the USA (Chen et al., 1995). Several qualitative and quantitative BSR genes have been reported (Von Wettstein-Knowles, 1992; Chen et al., 1994; Thomas et al., 1995; Hayes et al., 1996; Toojinda et al., 2000; Castro

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Published in Crop Sci. 43:651–659 (2003).

et al., 2002a, 2002b). We have focused our efforts on QR available in ICARDA/CIMMYT germplasm, using QTL mapping strategies (reviewed by Hayes et al., 2001). BSR resistance QTL from different germplasm accessions mapped on different chromosomes, allowing us to pyramid them in a new genetic background.

The pyramids of QTL alleles provide an independent validation of QTL effects detected in mapping populations and allow for estimation of QTL interactions. Genotypic information is essential for pyramiding QR genes because differential isolates of the pathogen cannot be used to determine the resistance allele architecture of the host, if the resistance is nonrace specific. Because the QR genes that were the targets of this research have not been cloned, we inferred allelic architecture on the basis of molecular markers linked to resistance QTL.

We developed genotypes combining two BSR resistance QTL alleles from the accession "Calicuchima-sib" on chromosomes 4 (4H) and 7 (5H) (Chen et al., 1994) (hereafter called QTL4 and QTL7), and a BSR resistance QTL allele from the cultivar Shyri on chromosome 5 (1H) (Toojinda et al., 2000) (hereafter called QTL5). Preliminary evaluation of these genotypes found that the QTL alleles act in an additive fashion such that genotypes with the most QR alleles have the highest levels of resistance under field epidemic conditions (Castro et al., 2000).

The principal thrust of our QR mapping efforts has been on adult plant resistance under field conditions, but we are also interested in QR QTL effects at the seedling stage. The growth stage at which resistance is expressed has important implications, on the basis of the experience gained in the wheat: wheat stripe rust pathosystem (Qayoum and Line, 1985). In this pathosystem, when a plant is exposed to the same virulence throughout its life cycle and it is resistant at all growth stages, it is defined as having "seedling resistance." In contrast, a plant exposed to the same virulence throughout its life cycle that is susceptible at the seedling stage but expresses resistance at later growth stages is defined as having "adult plant resistance." This adult plant resistance is often temperature dependent, and accordingly has been described in the literature as high temperature adult plant (HTAP) resistance (Line and Chen, 1995). Recently, HTAP resistance has also been reported in the barley: barley stripe rust pathosystem (X. Chen, unpublished data). HTAP in wheat is durable and often shows quantitative inheritance whereas seedling resistance is often not durable and shows qualitative inheritance (Chen and Line, 1995a,b).

In mapping populations, some QTL conferring resistance at the seedling stage map to the same chromosomal regions as QTL conferring resistance at the adult plant stage. In the Calicuchima-sib/Bowman population adult plant stage resistance QTL on chromosomes 4(4H) and 6(6H) were coincident with seedling stage resistance QTL (Castro et al., 2002b), while in the Shyri/Galena population adult plant stage QTL on chromosomes 5 (1H) and 6 (6H) were in the same chromosomal regions as seedling stage resistance QTL (Castro et al., 2002a). The chromosome 6 (6H) QTL was coincident in the two populations. QTL coincidence can be due to linkage or pleiotropy and in these mapping population studies adult stage plant resistance was measured under field conditions in response to local inoculum whereas seedling stage resistance was measured under controlled environment conditions in response to inoculation with defined isolates. Therefore, finer structure mapping and additional experiments will be necessary to determine if the same or different genes determine resistance at the seedling and adult plant stages and how these resistance QTL should be classified according to the HTAP nomenclature regarding "seedling resistance" and "adult plant resistance."

The QR QTL pyramid experiment that is the subject of this report was initiated before the discovery of the role of the 6(6H) QTL in seedling stage resistance and as a consequence this region of the genome was not monitored during population development. Our objectives were, accordingly, to determine if resistance at the seedling stage could be achieved in the QR QTL pyramids involving QTL4, QTL5, and QTL7. If resistance at the seedling stage was conferred by alleles at these QR QTL, we were interested in the nature of the QTL interaction.

MATERIALS AND METHODS

Plant Materials

A population of 115 doubled haploid (DH) lines (Chen and Hayes, 1989) was developed from the cross Harrington*2/Orca/2/D1-72 (Fig. 1). Harrington is a two-rowed malting barley cultivar developed by the University of Saskatchewan. Orca is a two-rowed barley cultivar obtained from the cross of Calicuchima-sib and Bowman, and has resistance alleles at QTL4 and QTL7 tracing to Calicuchima-sib (Hayes et al., 2000b; Castro et al., 2002b). D1-72 is a line from the Shyri/Galena population that has a resistance allele at QTL5 tracing to Shyri (Toojinda et al., 2000; Castro et al., 2002a). One cycle of marker-assisted selection was performed for resistance alleles at QTL4 and QTL7 in the BC_1 generation (Harrington*2/Orca). Four BC_1 plants with Orca alleles at marker loci flanking QTL4 and QTL7 were crossed with D1-72. DH lines were derived from the F_1 plants of these crosses, following the procedures described by Chen and Hayes (1989).

Phenotyping

The parents (Orca, Harrington and D1-72) and the DH progeny were assayed for resistance to BSR, at the seedling stage, in two experiments following the procedures described by Chen and Line (1992) and Hayes et al. (1996). In one experiment, conducted by X.M. Chen at Washington State University, three isolates were used. These isolates correspond to races PSH-13, PSH-14, and PSH-31 of *P. striiformis* f. sp. *hordei* and represent a range of virulence (Table 1) (Chen et al., 1995; Chen and Line, 2001). For each isolate test, 10 seeds of each parent and DH line were planted in a 10- by 10-cm plastic pot and grown in a rust-free greenhouse at a diurnal temperature cycle of 10 to 25°C. At the two-leaf stage, the seedlings were uniformly inoculated with urediospores mixed with talc (1:20 v/v), placed in a dew chamber at 10°C for 24 h, and then transferred to a glass-enclosed growth chamber within a greenhouse. The diurnal temperature cycle was gradually changed from 4°C at 0200 h to 20°C at 1400 h. A 16-h light/8-h dark photoperiod was provided by natural daylight

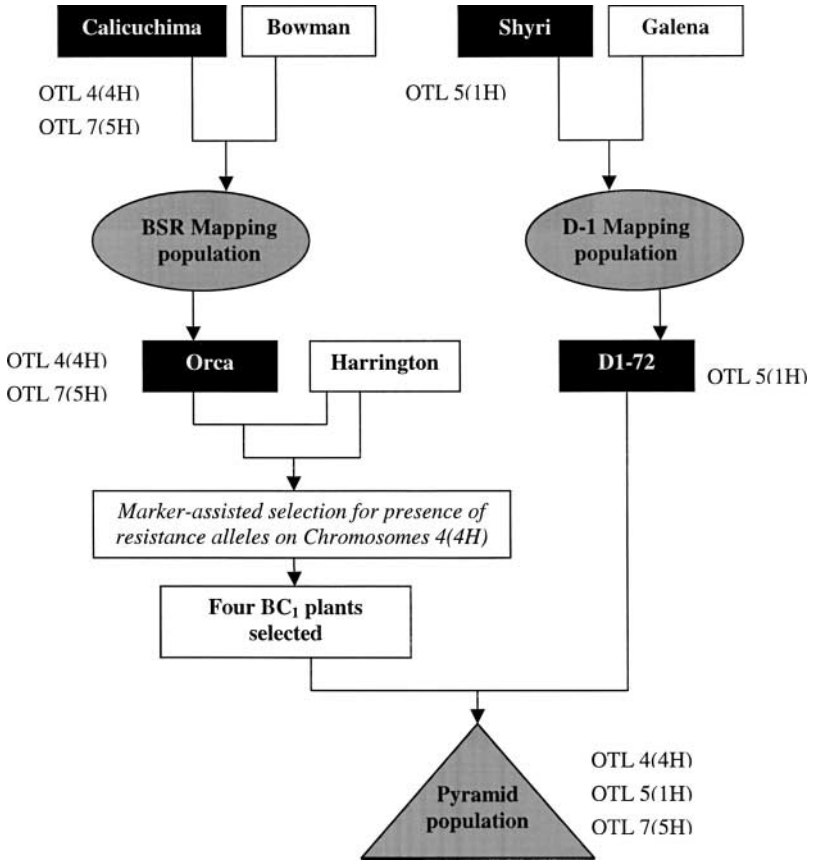

Fig. 1. Development of the barley stripe rust resistance QTL allele pyramid population. Black boxes represent resistance sources and white boxes represent susceptible parents. All of the mapping populations were composed of completely homozygous doubled-haploid lines.

supplemented with metal halide lights. The initial source of inoculum was urediospores that had been stored in liquid nitrogen. Each race was originally purified by single spore isolation or by transfer of single uredia for several generations. Data on infection type for each of the 10 plants were recorded 21 to 25 d after inoculation.

In another experiment, conducted by M. Johnston at Montana State University, an isolate of *P. striiformis* f. sp. *hordei* collected in Montana in 1997 (hereafter called BSTR-97) was used (Table 1). Ten seeds of each DH line were planted in a 10- by 10-cm pot. The plants were grown in a growth chamber with a 14-h light/10-h dark photoperiod provided by fluorescent lights and at a 15/20°C (dark/light) temperature regime. Seedlings were inoculated when the second leaf emerged, 10 to 12 d after planting, using a mixture of 3 mg of rehydrated lyophilized spores and 9 mg of talc. Plants were placed in a dew chamber at 10°C for 24 h and then returned to growth chambers at the previously described temperature and photoperiod settings. The isolate was purified by repeated transfer of single uredia. Data on infection type were recorded 18 d after inoculation. In both the Washington and Montana experiments, the seedling reaction to inoculation was rated as an infection type on the basis of a 0-to-9 scale (where 0 = complete resistance and 9 = complete susceptibility) as described by Line et al. (1974). Any variation in infection type within a pot is due to experimental error or mechanical mixture, because all test materials are doubled haploid lines or selfed seed of pure line cultivars. In the few cases where there were variable infection types within a pot, infection type corresponding to the majority of plants was used as the value for the genotype. Following the conventions of Line et al. (1974), Line and Qayoum (1991), and Chen and Line (1999), DH lines with infection type scores of 0 to 5 were classified as resistant (1) and DH lines with scores of 6 to 9 were classified as susceptible (0). Accordingly, all subsequent analyses were conducted based on these qualitative 1:0 ratings. The suitability of this conversion of infection type was further justified by the phenotypic frequency distributions for infection type shown in Fig. 2, which showed that there were few intermediate infection types that could have been incorrectly classified as resistant or susceptible.

Genotyping

The DH lines were genotyped in the regions defining the BSR QR QTL on chromosomes 4 (4H), 5 (1H), 6 (6H), and

Table 1. Virulence and avirulence of races PSH-13, PSH-14, PSH-31 (Chen and Line, 2001), and isolate BSTR-97 on a differential set of barley genotypes (S: susceptible, R: resistant, I: intermediate).

Differential					
No	Genotype	PSH-13	PSH-14	PSH-31	BSTR-97
1	Topper	S	S	S	S
2	Heils Franken	S	S	S	R†
3	Emir	R	S	S	I-S
4	Astrix	R	S	S	S
5	Hiproly	R	S	S	S
6	Varunda	S	S	S	R
7	Abed Binder 12	R	S	S	S
8	Trumpf	S	S	S	R
9	Mazurka	S	R	S	R
10	Bigo	S	S	S	R
11	I 5	R	R	R	R

† Sometimes gives inconsistent reactions.

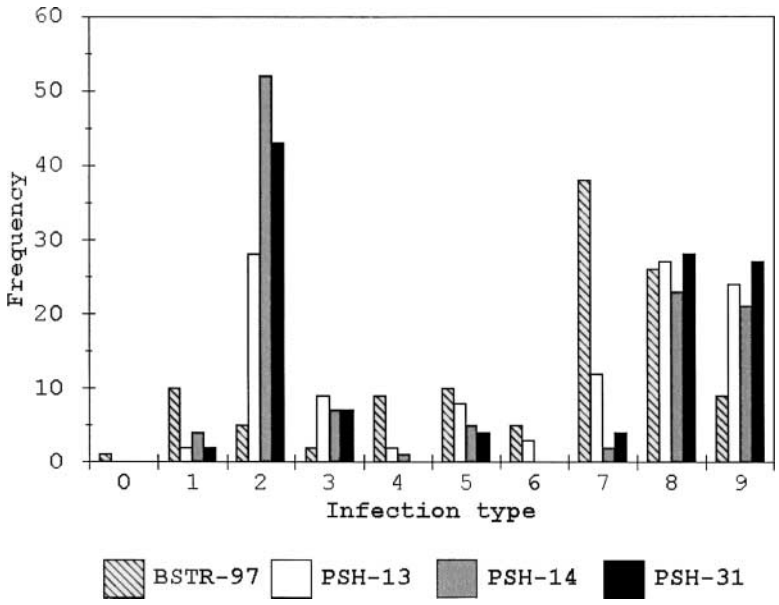

Fig. 2. Phenotypic distribution of infection type in the barley stripe rust resistance QTL pyramid population when inoculated at the seedling stage with four different isolates of *Puccinia striiformis* f. sp. *hordei*.

7 (5H) by means of 14 SSR markers. We first screened markers of known map position on Orca, Harrington, and D1-72 for polymorphisms. As our interest was to define the regions of chromosomes 4 (4H) and 7 (5H) that were introgressed from Orca, map positions of markers in these regions were confirmed in the Cali-sib × Bowman mapping population. In chromosome 5 (1H), our interest was in the region tracing to Shyri (via D1-72), and the reference population for confirming map position of polymorphic markers was Shyri × Galena. Neither Harrington nor D1-72 are known to carry resistance alleles at the QTL region on chromosome 6 (6H) that was important for seedling stage resistance in the Cali-sib/Bowman and Shyri/Galena mapping populations (Castro et al., 2002a,b). As shown in Fig. 3, we used three SSR markers (*EBmac788*, *HVMLO3*, and *HvAmyB*) for genotyping the chromosome 4 (4H) QTL region, four SSRs (*GMS21*, *Bmac213*, *Bmac399*, and *Bmac90*) for genotyping the chromosome 5(1H) region, and six SSRs (*Bmac303*, *Bmac337*, *HVM30*, *EBmac970*, *Bmac113*, and *Bmac223*) for the chromosome 7 (5H) region. We also used one SSR (*Bmag173*) for the chromosome 6 (6H) region. *Bmag-173* maps to the seedling stage resistance QTL peak in both original mapping populations. On the basis of marker allele genotypes, we were able to infer the presence or absence of the corresponding QR loci alleles in each of the lines. The SSR primer sets were developed and mapped by Ramsay et al. (2000), Pillen et al. (2000), Liu et al. (1996), and Becker and Heun (1995). For the SSR genotyping reverse primers were labeled with FAM, TET, NED, or HEX fluorescent dye. DNA amplifications were performed with either a Perkin-Elmer 9600 (Foster City, CA) or MJ Research PTC-100 (Waltham, MA) thermal cycler. PCR reactions were performed in a 10-μL reaction mix containing 37.5 ng of template DNA, 1× PCR buffer, 0.025 units *Taq* DNA polymerase (Qiagen, Valencia, CA), 0.2 n*M* dNTPs and 0.1 pmol of forward and reverse primers. Information on primer sequences and PCR amplification conditions for each set of primers is available at http://www.scri.sari.ac.uk/ssr (verified 26 September 2002) (Ramsay et al., 2000), in Liu et al. (1996) and in Becker and Heun (1995). PCR-amplified fragments from differentially labeled SSR primers and with nonoverlapping fragment sizes were simultaneously analyzed in the same gel lane and separated on an ABI Prism 377 DNA Sequencer at the Oregon State University (Central Service Lab) or on an ABI Prism 3700 DNA Sequencer at OMIC, Inc., Portland, OR. Gene Scan and Genotyper Software (Applied Biosystems, Perkin Elmer, Foster City, CA) were used for automated data collection and to determine the allele sizes in base pairs, based on the internal standard.

Data Analyses

The DH population structure (as it was derived from a complex cross) precluded the use of QTL analysis tools based on interval mapping. Accordingly, we used a QTL analysis analogous to candidate gene analysis, where the genotypes at marker loci are used as independent variables. Therefore, the independent variables had two levels each, with one level corresponding to the resistance allele (tracing to Orca in chromosomes 4 (4H) and 7 (5H), and tracing to D1-72 in chromosome 5 (1H)) and the other level corresponding to the alternative alleles at these QTL. There were no lines recombinant for the markers used to characterize QTL 4 or QTL 7 and as a consequence all DH lines were included in analyses of these QTL. One DH line was recombinant for QTL7 markers; accordingly analysis of this QTL was based on 114 lines. The treatment design was a $2 \times n$ factorial, where n is the number of genome regions targeted. The difference between parental marker class means estimates the additive effect of the QTL. Double crossovers between the QTL and marker loci would downward bias estimates of QTL effects. Thus, differences between parental marker genotype means are conservative estimates of the effects of QTL residing in the chromosomal regions under study. Because the response (dependent) variable was binomial (1 = resistant and 0 = susceptible) and the response probability distribution was binomial, the analysis was performed using a generalized linear model with a logit link function and binomial errors.

Statistical analyses were performed by the SAS (2001) GENMOD procedure. Parameters and test statistics were estimated by a Type III analysis (analogous to partial sums of squares analyses of general linear models). We performed separate analyses of the effects for each stripe rust race and a combined analysis using the four isolates. The analyses of each individual isolate entailed estimating the least squares

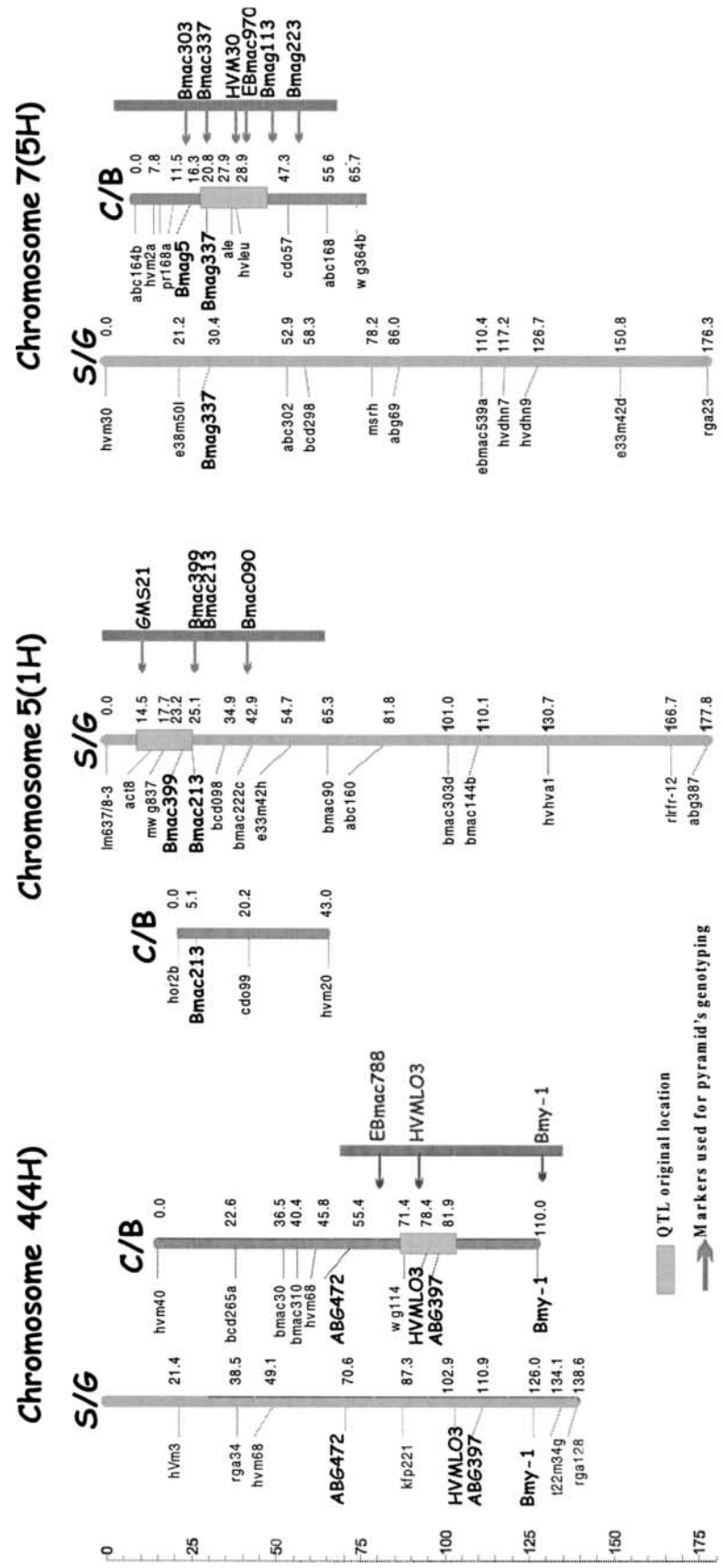

Fig. 3. Alignment of the original mapping populations for the target regions on chromosomes 4(4H), 5(1H), and 7(5H), showing the markers used for genotyping the barley stripe rust resistance QTL pyramid population. "C/B" refers to Calicuchima/Bowman, "S/G" refers to Shyri/Galena. Markers indicated with larger font in the original maps were included to facilitate map alignment. *Bmy1* and HvAmyB are different PCR-based markers derived from β *amylase-1* allele sequences.

Table 2. Numbers of resistant (R) and susceptible (S) barley doubled haploid lines when inoculated at the seedling stage with four different isolates of *Puccinia striiformis* f. sp. *hordei*, classified according to reaction to each isolate.

Isolate				
BSTR-97	PSH-13	PSH-14	PSH-31	No. of DH lines
R	R	R	R	27
R	R	R	S	1
R	R	S	R	0
R	R	S	S	0
R	S	R	R	2
R	S	R	S	5
R	S	S	R	1
R	S	S	S	2
S	R	R	R	16
S	R	R	S	3
S	R	S	R	1
S	R	S	S	1
S	S	R	R	7
S	S	R	S	7
S	S	S	R	3
S	S	S	S	39
38	49	68	57	Total R lines
77	66	47	58	Total S lines

means for each QTL and their interactions, additive effects, additive by additive interaction effects, and likelihood ratio statistics for tests of significance of the effects (P values were calculated using asymptotic chi-square distributions). The joint analysis entailed estimating the least squares means and test statistics for the effect of stripe rust isolate (I) and interaction effects between I and QTL, in addition to the main and interaction effects across isolates. The probability of resistance to stripe rust was estimated for QTL allele genotypes by $e^p/(1 + e^p)$, where p is the least squares mean for the individual QTL and QTL × QTL interaction.

RESULTS

Ninety-eight of 115 lines (85%) had the same reaction to at least three of the isolates. Eighty-four of 115 lines had the same reaction to each of the three PSH isolates, 66 of these 84 lines also had an equivalent reaction to BSTR-97. There were fewer lines showing a resistant reaction to isolate BSTR-97, compared with reactions to the PSH isolates and more lines were resistant to PSH-14 than to any other isolate. The differential reactions of some DH lines (Table 2) could be due to the specificity of their resistance to the isolates, although there was a clear pattern of association of resistance vs. susceptibility alleles at QTL4 and QTL5 and seedling reaction to inoculation (Table 3). The absence of resistance alleles at both QTL4 and QTL5 was associated with the susceptible phenotype. The effects of resistance vs. susceptibility alleles were greatest in response to inoculation with PSH-14 and PSH-31. The likelihood ratio tests for each individual isolate and the joint analysis of all four isolates confirmed the importance of both QTL in relation to the seedling stage disease reaction phenotype (Table 4). In all cases, both QTL were significant and no significant race × QTL interaction was detected. However, there was a significant race effect that reflects the higher number of susceptible lines observed after inoculation with BSTR-97. Further experiments are necessary to determine the race specificity of the resistance QTL and thus to resolve their classification in terms of the seedling and adult plant resistance criteria used to define HTAP resistance.

Table 4. Likelihood ratios for tests of significance of QTL main and interaction effects from separate analyses of reaction of barley doubled haploid lines when inoculated with each of four stripe rust isolates. Also shown are QTL and QTL and isolate main and interaction effects for the combined analysis of all isolates. *P*-values were calculated using asymptotic chi-square distributions.

	Effect	χ^2 statistic	$P < \chi^2$
BSTR-97	QTL4	16.59	0.0010
	QTL5	8.30	0.0040
	QTL4*QTL5	0.98	0.1691
PSH-13	QTL4	20.02	0.0001
	QTL5	24.74	0.0001
	QTL4*QTL5	0.51	0.4769
PSH-14	QTL4	21.82	0.0001
	QTL5	8.86	0.0029
	QTL4*QTL5	1.55	0.2134
PSH-31	QTL4	26.64	0.0001
	QTL5	23.39	0.0001
	QTL4*QTL5	1.06	0.3031
Joint analysis	QTL4	84.45	0.0001
	QTL5	59.05	0.0001
	QTL4*QTL5	4.66	0.0309
	Race	19.70	0.0002
	Race*QTL4	0.74	0.8627
	Race*QTL5	3.12	0.3739
	Race*QTL4*QTL5	0.20	0.9773

Table 3. Numbers of resistant (R) and susceptible (S) barley doubled haploid lines when inoculated at the seedling stage with different isolates of *Puccinia striiformis* f. sp. *hordei*. Infection types (0–9) are shown in parentheses. The lines are classified according to their reaction to each one of the isolates and according to the alleles present in the QTL regions on chromosomes 4(4H) and 5(1H).

		Isolate							
		BSTR-97		PSH-13		PSH-14		PSH-31	
Resistance allele architecture		No. of lines (and avg. infection type)		No. of lines (and avg. infection type)		No. of lines (and avg. infection type)		No. of lines (and avg. infection type)	
QTL4†	QTL5‡	R	S	R	S	R	S	R	S
+	+	14	7	18	3	19	2	20	1
		(2.8)	(6.9)	(2.1)	(8.0)	(1.9)	(6.5)	(2.1)	(7.5)
+	−	9	11	9	11	16	4	12	8
		(2.3)	(7.2)	(2.4)	(7.5)	(2.2)	(8.0)	(2.3)	(7.9)
−	+	13	27	20	20	27	13	23	17
		(3.8)	(7.6)	(3.3)	(8.0)	(2.3)	(8.2)	(2.4)	(8.2)
−	−	2	32	2	32	6	28	2	32
		(3.0)	(7.7)	(3.5)	(8.4)	(3.2)	(8.6)	(3.5)	(8.7)
Total number of lines		38	77	49	66	68	47	57	58

† + = resistance allele present; − = allele absent.
‡ Orca contributes the resistance allele in QTL4; D1-72 contributes the resistance allele in QTL5.

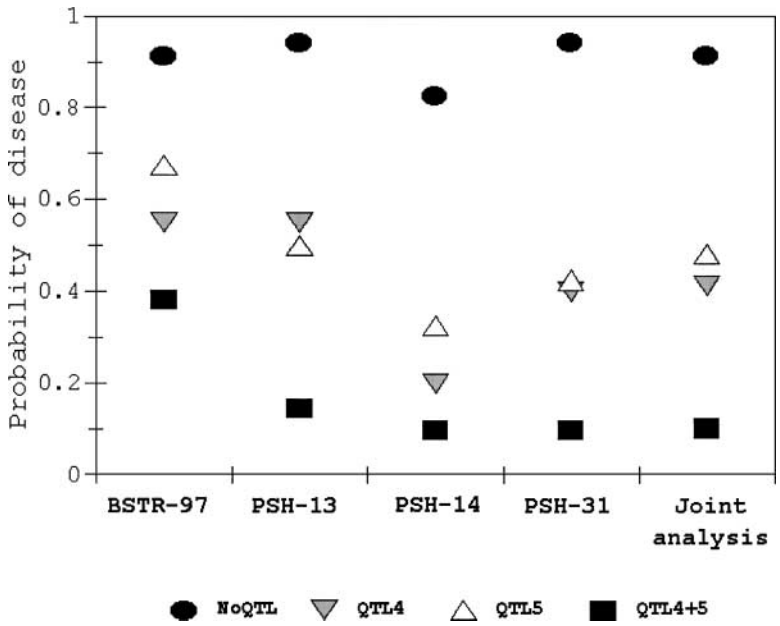

Fig. 4. Least squares means of the probability of occurrence of the susceptible phenotype in individuals with resistance alleles on QTL4, QTL5, and both QTL4 and QTL5, and with no resistance alleles. Data are shown for inoculation with each of four stripe rust isolates and for all isolates considered jointly.

We also assessed the role of QTL7 and the QTL on chromosome 6(6H) in the expression of resistance at the seedling stage to the four isolates. QTL7 was not a significant determinant of resistance at this growth stage in the original mapping population (Castro et al., 2002b), but because it was a significant determinant of QR in field studies at the adult plant stage (Chen et al., 1994) it was a target for MAS in the development of the DH lines used in this study (Fig. 1). No main effect of QTL7 or QTL7 × race interaction was detected (data not shown). Regarding chromosome 6(6H), which was a significant determinant of resistance at the seedling stage in the two source mapping populations (Castro et al., 2002a, 2002b), D1-72 lacks the Shyri (resistance) alleles at this QTL, and the genotyping with *Bmag173* did not detect Orca alleles. Because the region was not targeted in the MAS scheme, the absence of Orca alleles means that the four BC_1 plants selected for the final cross carried Harrington alleles at this QTL. Harrington is not known to carry any BSR resistance alleles. This cultivar is highly susceptible to the four isolates (data not shown).

As shown in Fig. 4, the probability of a susceptible phenotype was lower than 10% when resistance alleles were present at both QTL (for the joint analysis), and higher than 80% in all analyses when resistance alleles were absent at both QTL. The presence of resistance alleles at only one QTL was associated with intermediate probabilities of disease occurrence.

DISCUSSION

Our previous findings regarding the effects of QTL4 and QTL5 on resistance at the seedling stage as measured under controlled environment conditions and resistance detected at the adult plant stage under field conditions (Hayes et al., 1996; Castro et al., 2002a,b) have implications for a range of host plant resistance issues, with particular reference to the relationship between quantitative and qualitative resistance and the growth stage specificity of disease resistance. The results presented herein further address these issues and raise additional questions of interest.

Our results validate our earlier reports that resistance alleles are necessary at more than one QTL to increase the likelihood of the resistant phenotype being expressed at the seedling stage (Castro et al., 2002a,b). Alleles at QTL4 and a QTL on chromosome 6(6H) were necessary for resistance at the seedling stage in the Calicuchima × Bowman mapping population, whereas alleles at QTL5 and a QTL on chromosome 6(6H) were necessary in the Shyri × Galena mapping population. In both cases, the resistance fit a complementary gene model: two QTL were necessary to have a probability of 80% or higher of recovering the resistant phenotype. In the current study, we also find that, in a new genetic background, resistance alleles at two QTL are necessary to recover the resistance phenotype at a high level of probability. In terms of allele interaction in a new genetic background, we found significant QTL × QTL interaction in the joint analysis of the four isolates. As shown in Fig. 4, only in the case of PSH-14 did alleles at a single QTL have a high probability of resistance and the highest number of resistant lines was observed after inoculation with this isolate. As is evident in Table 3, there were lines showing a resistant phenotype that had only a single resistance allele, at either QTL 4 or QTL 5. However, except for isolate PSH-14, there were also as many, or more, susceptible lines with the same allelic architecture. Thus, while an individual QTL allele is necessary for resistance, it is not sufficient. There are also a few lines with no QTL resistance alleles that are resistant and lines with both QTL resistance alleles that are susceptible. This may be due to double

Table 5. Comparisons of least squares means for the probability of occurrence of the susceptible phenotype, given specified QTL resistance allele configurations, after inoculation with specified isolates. Data are based on the original QTL mapping reports and from the doubled haploid barley stripe rust resistance QTL pyramid population.

	Isolate	Original report†	Pyramid population
QTL4	BSTR-97	0.467	0.550
QTL5	PSH-13	0.665	0.500
	PSH-14	0.667	0.325
Two QTL‡	BSTR-97	0.036	0.381
	PSH-13	0.214	0.143
	PSH-14	0.214	0.095
No QTL	BSTR-97	0.667	0.912
	PSH-13	1.000	0.941
	PSH-14	1.000	0.824

† Castro et al. (2002b) for BSTR-97 and QTL4; Castro et al. (2002a) for PSH-13, PSH-14 and QTL5.

‡ For BSTR-97, in the original report the two QTL were QTL4 and a QTL on chromosome 6(6H). For PSH-13 and PSH-14, in the original report the QTL were QTL5 and another QTL on chromosome 6(6H). For the pyramid population the QTL are QTL4 and QTL5.

crossover between markers and disease resistance loci, undetected resistance genes, and/or incomplete penetrance.

The results of this study validate that resistance alleles at QTL4 and QTL5 are associated with BSR resistance at the seedling stage and that the presence of resistance alleles at both loci substantially increases the probability of recovering the resistant phenotype. These results also validate the coincidence of QTL for resistance at the adult plant stage under field conditions and seedling stage resistance under controlled environment conditions as reported by Castro et al. (2002a,b). A comparison of the probabilities of recovering the resistant phenotype in the source mapping populations and in the derived DH lines confirms the consistency of effect of these seedling stage resistance QTL (Table 5). However, in the mapping populations, a resistance allele at a QTL on chromosome 6(6H) was necessary for resistance, in conjunction with a resistance allele at either QTL4 or QTL5. In the DH lines studied in this experiment, resistance alleles were also necessary at two QTL, but the two QTL are on chromosomes 4(4H) and 5(1H). In other words, an allele at QTL4 or QTL5 can substitute for an allele on chromosome 6(6H). We are currently developing a set of lines isogenic for BSR resistance QTL to determine the resistance mechanisms associated with each QTL. The results of experiments involving these isogenic lines inoculated with multiple isolates at multiple growth stages should be of assistance in understanding how the number of QTL resistance alleles relates to resistance.

These isogenic lines should also help to resolve the issue of race specificity of QTL. At the current level of resolution it is not possible to know if coincident resistance QTL represent the effects of the same genes or linked genes. The QTL alleles we targeted comprise large genome regions, averaging 20 centimorgans (cM). Estimates of physical (Mb, megabases) to genetic (cM) ratios for these regions of the genome range from less than 1.0 to more than 4.4 Mb/cM (Künzel et al., 2000; Hayes et al., 2000a). Resistance genes are known to cluster in plant genomes (Michelmore, 1995; Hulbert et al., 2001), so it is also possible that coupling linkages of multiple resistance alleles could underlie the coincident QTL for resistance to the four isolates. At the level of resolution afforded by the current genotype data in this germplasm, QTL4 and QTL5 confer resistance to multiple isolates at both the seedling and adult plant stages and accordingly do not fit the definition for adult plant resistance, as defined in the HTAP model. Unfortunately, durability can only be demonstrated in hindsight.

The results of these experiments demonstrate the utility of the approach that we used to identify the genomic regions that affect a binomially distributed trait, such as seedling resistance to BSR. QTL mapping tools were developed to deal with normally distributed variables (Doerge, 2002), and may not be appropriate for nonnormal variables. In a previous report (Castro et al., 2002a) we proposed and applied a three-step approach using chi-square tests, QTL interval mapping, and candidate gene analysis using generalized linear models to detect the seedling stage resistance QTL in the reference mapping populations.

The first generation of QTL analyses lent support to the idea that relatively few genetic factors were found to be the principal determinants of complex traits (Dekkers and Hospital, 2002). However, the initial enthusiasm for QTL analysis and the potential gains to be achieved by MAS has been tempered by concerns regarding bias in QTL estimation (Beavis, 1998), a paucity of studies validating QTL alleles in different genetic backgrounds and few examples of successful MAS (Dekkers and Hospital, 2002). The small population sizes typically used for QTL detection lead to overestimation of QTL effect and underestimation of QTL number and interaction (Melchinger et al., 1998), in which case, the prospects for MAS are not encouraging (Bernardo, 2001).

We have generated data that lead us to support the application of QTL analysis and MAS for QR. In the case of seedling and adult plant stage resistance to BSR, results of agricultural significance can be achieved by QTL information. A comparison of QTL effects as estimated in the source mapping populations and in the derived lines reveals changes in magnitude of effect. This could be due to bias in estimation of QTL effects in the source mapping populations, or it could be due to uncharacterized interactions of the resistance QTL alleles with the new genetic background. In any event, QTL mapping and marker information were useful for the characterization of BSR resistance gene pyramids.

ACKNOWLEDGMENTS

This research was supported by the North American Barley Genome Project, USDA-ARS, American Malting Barley Association, Oregon Grains Commission, Idaho Barley Commission, and the Washington Barley Commission.

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